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VIAL WITH ACTIVATED PROTEIN C

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[There are no amendments to this patent.]

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Abstract

Objective

A vial filled with a 24-h dose of activated protein C.

Constitution

A vial containing 2000-20000 units of activated protein C.

Claims

1. A vial with activated protein C containing 2000-20,000 units of activated protein C.

2. A vial with activated protein C containing 2000-20000 units of activated protein C, 250-1000 mg human blood serum, 60-240 mg sodium citrate, 50-200 mg glycine, and 70-280 mg sodium chloride.

Detailed explanation of the invention

[0001]

Industrial application field

The present invention is concerned with a drug form for intravenous injection of activated protein C possessing anticoagulant activity.

[0002]

Prior art

Protein C is a vitamin K-dependent glycoprotein. This glycoprotein is synthesized in the liver, and circulates in blood plasma as a proenzyme at a concentration of about 4 μg/mL. Protein C is converted by thrombin-thrombomodulin complex on vascular endothelium into active serine protease, in other words, activated protein C (hereinafter abbreviated APC). Because APC carries out limited hydrolysis of the proteins of both factor Va, which is a cofactor of prothrombin activation (thrombin production) induced by Factor Xa, and factor VIIIa, which is a cofactor of factor X activation induced by factor IXa, it possesses anticoagulant effects.

[0003]

Because APC suppresses the tPA-inhibiting activity of tPA inhibitors by binding with the corresponding tPA inhibitor, it also possesses fibrinogenolysis-promoting activity.

[0004]

Problems to be solved by the invention

The authors of the present invention have conducted investigations into injection of protein C in an activated form, in other words, APC, for the treatment of several types of thrombosis, as well as in-depth studies to determine the drug

form, in which a 24-h dose is contain d in a single ampul so as to be able to perform injections in a simple op ration.

[0005]

Means to solve the problems

The present invention consists of:

1. A vial with activated protein C containing 2000-20000 units of activated protein C, and
2. A vial with activated protein C containing 2000-20000 units of activated protein C, 100-1000 mg human blood serum, 24-240 mg sodium citrate, 20-200 mg glycine, and 28-280 mg sodium chloride.

[0006]

The activated protein C used in the present invention is w ll-known in the technical field. This may be a protein obtained in vitro by activating protein C (hereinafter abbreviated as PC) from blood or prepared by a genetic recombination technique with thrombin or thrombin-thrombomodulin complex, or a protein obtained by direct expression as APC using a genetic recombination technique.

[0007]

A unit of such APC is defined as the amount that doubles th activated [partial] thromboplastin time (APTT) of normal human blood plasma, and is specifically measured in the manner described below.

[0008]

The APC activity measurement method consists of measuring the APTT (seconds) of a diluted sample by adding normal human blood plasma, and using the dilution ratio obtained at the moment when this value is two times the value of the control (buff r solution) as the value of APC activity of the sample.

[0009]

Procedure

A sample is diluted with a buffer solution of 1% HSA and Veronal (for example, 400, 500, 800, 1000 times). 100 µL of normal human blood plasma (for example, cytolol [transliteration] and 100 µL of APTT reagent (for example, actin) are added at an interval of 15 sec and mixed with 100 µL of diluted solutions of samples or control (buffer solution) at 37°C, and 100 µL f 0.025M $CaCl_2$ are added 2 h later, measuring the coagulation tim .

[0010]

Calculation of activity

The APTT values (Y) obtained at the dilution ratios (X) of the control and samples are used to obtain and an equation of linear regression of Y a coefficient of correlation dependent on $10^3/X$.

[0011]

$Y = A(10^3/X) + B$, and,
if the doubled value of the APTT (in seconds) of th control is designated as Y_1, then the APC activity of the samples (unit: mL) is the value of X_1 obtained from:
$X_1 = 10^3\{(Y_1 - B)/A\}$.

[0012]

Vials used for drip and regular intravenous injections in m dical facilities, for example, those recorded in the Japanese Pharmacopoeia, can be used as the vials for storage of 2000-20000 units of such activated protein C.

[0013]

Although APC may be contained in such vials in a pure form, usually it is mixed with various stabilizers in order to ~~stabilize APC~~. Proteins and various salts, as well as amino acids used alone or in combination are representative of such stabilizers, with the proteins selected from albumin, immunoglobulin, salts selected from sodium chloride, citrates, and phosphates. Glycine, lysine, and alanine are suggested as the amino acids.

[0014]

Among such combinations, combinations of sodium chloride, glycine, sodium citrate, and human blo d serum albumin are particularly suitable.

[0015]

When the vial with activated protein C of the present invention is used for intravenous injections and drip, 4-40 cc of physiological saline or purified water for injection are added per vial of the present invention, and if necessary for intravenous drip, it may be further dissolved in 200-1,000 cc of water used for intravenous drip. Because a single vial of th present invention constitutes a 24-h dose, there is no need to mix the contents of several vials or to divide the contents of single vials into multiple portions. For this reason, because there can be no dosage errors, and because no painstaking mixing and division operations are required, it can be used in a simple and easy manner in a medical facility.

[0016]

Below, the present invention is explained in greater detail by referring to application examples.

[0017]

Application Example 1

M thod of APC preparation

As an example of a method for the preparation of APC used in th present invention, a method is shown below in which APC is obtained by activating PC prepared from normal human blood plasma with thrombin. Namely, protein C (PC) is isolated by subjecting normal human fresh frozen plasma to affinity chromatography using monoclonal antibodies recognizing the Ga-domain. It is activated with thrombin, subjected to chromatographic refining using an anion-exchange substance, and then, upon dialysis treatment, to filtration, and freeze-drying. See specific examples in the application "Human activated protein C preparations and pr parative method therefor" (Japanese Patent Application No. Hei 5[1993]-292499) filed by the authors of the present application on October 29, 1993.

[0018]

An APC preparation of the composition shown below is prepared by adding a stabilizer to the thus-obtained APC. See specific examples of the preparative method in the application "Method of stabilization and stabilizing composition for protein C or activated protein C" (Japanese Patent Application No. Hei 05[1993]-292500) filed by the authors of the present application on October 29, 1993.

[0019]

Table I

Activated protein C (APC)	500 units
Human blood serum albumin	25 mg
Sodium citrate	6 mg
Glycine	5 mg
Sodium chloride	7 mg

[0020]

The product is sealed in vials in an amount of 2000-20,000 units of APC.

[0021]

Application Example 2

Experiment for determining units of administration

48 patients with leukemia accompanied by DIC were divided into 3 groups, administered, respectively, 50 units, 150 units and 300 units/kg·day APC every day and checking their overall improvement and general stability. The overall improvement was optimum at 300 units/kg·day, and there were no problems with general stability either.

[0022]

Dosages from 150-300 units/kg·day can be used in the futur .

[0023]

It is preferabl to prepare a series of vials c ntaining from 1500-21,000 units/vial, and preferably, from 2000-20,000 units/vial as a 24-h dose per 10-70 kg of body weight of children and dults.

[0024]

Application Example 3

A 23-year-old female patient Y.I. (weight: 50 kg) with acute lymphocytic leukemia as the basic disease was diagnosed with disseminated intravascular coagulation (DIC), with a DIC score of 4 points. The patient was subjected to intravenous drip with APC in the amount of 300 units/kg/day for 6 days using vials containing 15,000 units of APC each. The DIC score on the last day of drip was 2 points, with a medium improvement based on the DIC score. Also, improvement based on FM testing was considered a conspicuous improvement. Based on this, the global evaluation was that the treatment was efficient with respect to DIC, there were no side effects, and the administration was extremely us ful.

[0025]

Application Example 4

The patient was a 72-year-old male. He had been under observation for protein C (PC) deficiency syndrome, high blood

pressure, and abdominal aneurysm since Heisei X_1*. An increas in the aneurysm was observed since March Heisei X_2, and he was admitted to the hospital for thorough examination and treatment. A fusiform aneurysm accompanied by thrombosis extending from right below the renal artery to the common iliac vein furcation region was found, and from the coagulopathic standpoint, there was concern that DIC could be also present. PC activity was 48%, and the amount of antigen dropped as low as 35%, while the s cond son of the patient was also diagnosed with type I PC deficiency with similar low PC activity and amount of antigens. While controlling DIC with heparin in an amount of 10,000 units/day and Chiguropijin [transliteration] in an amount of 200 units/24 h, during angiography conducted before the operation and during th operation, the patient was given a continuous drip infusion of an activated PC preparation (CTC-111) in the amount of 200 units/kg/24 h, which allowed performance of an aneurysm excision operation safely without thrombosis. An improvement in DIC was observed 10 days after the operation. Supplementation with the activated PC preparation during the operation to remove an abdominal aneurysm accompanied by serious DIC in a patient with a PC deficiency as a basic disease proved successful.

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* Translator's note: since 1989 or later